

Claims:

1. Highly active glycoprotein produced by a process comprising:
 - 5 expression of said highly active glycoprotein in an expression cell line, harboring at least one defect in the sugar nucleotide biosynthetic pathway of sialic acids and which is transfected with nucleic acid encoding the glycoprotein, in a medium supplemented with a concentration of at least one sialic acid precursor additive, the concentration being determined by a process comprising:
 - 10 (i) expression of a plurality of different sialylation forms of said glycoprotein by differential sialylation using different concentrations of at least one sialic acid precursor;
and
 - (ii) determination of the activity of the different sialylation forms in comparison
15 with a reference glycoprotein in (a) suitable bioassay(s);
and
 - (iii) selection of the sialylation form with the higher/highest activity and determination of the concentration of the sialic acid precursor additive(s) which is correlated with the higher/highest activity level of said
20 glycoprotein.
2. A glycoprotein according to claims 1, wherein the glycoprotein is secreted by the cells of the expression cell line.
- 25 3. A glycoprotein according to claim 1 or 2, wherein only one sialylation form is expressed in step (i).
4. A glycoprotein according to at least one of claims 1 to 3, wherein the higher activity of the glycoprotein is characterized by a higher activity in at least one in vitro model and/or a
30 higher activity in at least one in vivo model and/or a higher stability and/or a longer serum-half life and/or a longer bioavailability and/or an improved immunogenicity and/or an improved antigenicity determined by at least one bioassay.
5. A glycoprotein according to at least one of claims 1 to 4, wherein the defect in the
35 biosynthetic pathway of sialic acid is a mutation of an epimerase.

6. A glycoprotein according to at least one of claims 1 to 5, wherein the expression cell line is NM-F9 or NM-D4.
7. A glycoprotein according to at least one of claims 1 to 6, wherein the glycoprotein is selected from the group comprising Glycophorin A, EPO, G-CSF, GM-CSF, FSH, hCG, LH, interferons, interleukins, antibodies and/or fragments thereof .
8. A glycoprotein according to at least one of claims 1 to 7, wherein at least one sialic acid precursor additive is ManNAc, acetylated ManNAc, peracetylated ManNAc or fetuin.
9. Process for the production of a highly active glycoprotein according to at least one of claims 1 to 8 comprising:
expression of said highly active glycoprotein in an expression cell line, harboring at least one defect in the sugar nucleotide biosynthetic pathway of sialic acids and which is transfected with nucleic acid encoding the glycoprotein, in a medium supplemented with a concentration of at least one sialic acid precursor additive, the concentration being determined by a process comprising:
- (i) expression of a plurality of different sialylation forms of said glycoprotein by differential sialylation using different concentrations of at least one sialic acid precursor;
 - and
 - (ii) determination of the activity of the different sialylation forms in comparison with a reference glycoprotein in (a) suitable bioassay(s);
 - and
 - (iii) selection of the sialylation form with the higher/highest activity and determination of the concentration of the sialic acid precursor additive(s) which is correlated with the higher/highest activity level of said glycoprotein.
10. Process for the identification/determination of a highly active glycoprotein according to at least one of claims 1 to 8 comprising:

- i) transfection of expression cell line harboring at least one defect in the sugar nucleotide biosynthetic pathway of sialic acids with nucleic acid encoding the glycoprotein;
and
5 ii) expression of a plurality of different sialylation forms of said glycoprotein by differential sialylation using medium with different concentrations of at least one sialic acid precursor additive;
and
10 iii) determination of the activity of the different sialylation forms in comparison with a reference glycoprotein in (a) suitable bioassay(s);
and
iv) selection of the sialylation form with the higher/highest activity.
11. Process for differential sialylation of a glycoprotein according to at least one of claims 1
15 to 8, characterized in that, a plurality of different sialylation forms of said glycoprotein are expressed in an expression cell line, harboring at least one defect in the sugar nucleotide biosynthetic pathway of sialic acids and which is transfected with nucleic acid encoding the glycoprotein, by using medium with different concentrations of at least one sialic acid precursor additive.
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12. Process for the generation of an expression cell line with a defect in the sugar nucleotide biosynthetic pathway of sialic acids comprising the selection of expression cell line from primary cells or cell lines with a recognition molecule that binds to desialylated structures which can be sialylated by at least two enzymes.
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13. Process of claim 12, wherein the cells from primary cells or cell lines are mutagenized before selection.
14. Process of claim 12 or 13, wherein the structures are O-glycans.
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15. Process according to at least one of claims 12 to 14, wherein the desialylated structures can be sialylated by alpha2-3 and alpha2-6 bound sialic acids.
16. Process according to at least one of claims 12 to 15, wherein the recognition molecule
35 is a lectin or a carbohydrate specific antibody.

17. Process according to at least one of claims 12 to 16, wherein the recognition molecule is a lectin or a carbohydrate specific antibody recognizing the core-1 structure.
- 5 18. Process according to at least one of claims 12 to 17, wherein the expression cell line is derived from the group comprising Per.C6, HEK293, K562, CV1, COS-7, Hybridoma cells, Namalwa, BHK and CHO.
- 10 19. Composition for *in vitro* and *in vivo* use, comprising a glycoprotein according to at least one of claims 1 to 8, and a diluent or carrier.
20. Pharmaceutical composition for use in therapy, comprising a glycoprotein according to at least one of claims 1 to 8, and a pharmaceutically-acceptable diluent or carrier.
- 15 21. Pharmaceutical composition according to claim 20, characterized in that, the composition is a vaccine or vaccine-adjuvant.
22. Use of the glycoprotein according to at least one of claims 1 to 8 for the manufacture of a vaccine or vaccine-adjuvant for treatment of infectious diseases.
- 20 23. Use of the glycoprotein according to at least one of claims 1 to 8 for the manufacture of a medicament for prophylactic and/or therapeutic treatments of diseases selected from the group comprising leukemia, neutropenia, cytopenia, cancer, bone marrow transplantation, diseases of hematopoietic systems, infertility and autoimmune diseases.